A PORTABLE KIT FOR RAPID DIAGNOSIS OF INFECTIOUS

DISEASES UNDER FIELD CONDITIONS


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# A PORTABLE KIT for RAPID DIAGNOSIS
# of INFECTIOUS DISEASES under FIELD CONDITIONS

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## ABSTRACT

A portable kit has been developed for identifying infectious disease agents under field conditions, i.e., without laboratory facilities. The basic techniques employed are counterimmunoelectrophoresis, coagglutination, and diagnostic microscopy, including differential stains. This portable kit has been evaluated in various field and laboratory circumstances in tropical and temperate areas for rapid diagnosis of cerebrospinal meningitis, salmonellosis, and cholera. The carrying case, working components, and diagnostic reagents have withstood aircraft transport, baggage handling, international mailing, international air freight shipment, and rough road transport by Land Rover. It has performed at ambient temperatures ranging from $15^{\circ}$C to $43^{\circ}$C. By simply substituting other appropriate antisera, antigens, and reagents, this system is adaptable to diagnostic identification of numerous other infectious disease agents.

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## Introduction:

Infectious diseases comprise a major problem area in tropical medicine. They cause untold human suffering and are an important factor in retarding economic development of many countries.

Among the more common acute tropical infectious diseases are cerebrospinal meningitis, enteric fevers, gastroenteritis, and bacterial pneumonias. These diseases are endemic in many developing areas, and some also may occur in epidemic form.

Early, specific diagnosis of many infections is an important aid in curative and preventive medicine. It permits timely initiation of appropriate therapy and thus promotes return of patients to full occupational activity as soon as possible. In preventive medicine, knowledge of the exact etiology of infectious diseases is essential for tracing their source and mode of spread. Thus, specific etiologic diagnoses of many infections ultimately may lead to their prevention and control.

Many of the problems faced in tropical infectious disease control are primarily logistic. It is often difficult to bring patients and medical assistance together. This is due to the widely spread rural distribution of much of the population in many developing areas, as well as difficult terrain and transport in many situations. This is especially true with respect to laboratory services. In many instances, outbreaks of severe acute diseases occur in rural areas remote from laboratory facilities. Thus, the diagnostic information needed for specific treatment and control is often not available.

Approach:

A portable kit has been designed to facilitate rapid identification of infectious disease agents. Furthermore, the simple and rapid diagnostic techniques employed have been adapted to permit their use in the field. The objective is to provide specific etiologic diagnoses where laboratory facilities do not exist.

The portable diagnostic kit is compactly contained in a briefcase measuring 47 x 39 x 21 cm, and it weighs approximately 13 kilograms. Equipment and supplies in the kit are shock-protected, and reagents are temperature-protected by special insulation. The kit is easily carried and fits under the seats of commercial aircraft, thus enabling an epidemiologist to keep the diagnostic laboratory in personal custody while traveling. (Figure 1).

The sero-diagnostic tests used in this kit include the counterimmuno-electrophoresis (CIE) test and the coagglutination (COAG) test. The COAG test is incorporated as a small sub-kit that may be employed independently (Figure 2). Functionally similar tests such as latex agglutination can also be included to meet specific requirements.

In addition to serological test systems, standard light microscopy is frequently useful in rapid diagnosis. For example, Gram stain smears of cerebrospinal fluid, exudates, and lesions can yield very valuable diagnostic information. Blood and stool examinations for parasites require a microscope, as do examinations for certain superficial mycoses. The McArthur microscope, a compact optical instrument with a wide range of capabilities, has been included in the kit. This rugged instrument is highly suited to field work, since it can be operated in the hand (Figure 3) and carried independently if desired. Accessory materials and appropriate stains may be selected by the user to meet individual requirements. These can be included among the kit supplies in special dispenser bottles and compartments provided. (Figure 4).

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# COUNTERIMMUNOELECTROPHORESIS (CIE) TEST

Background:  The CIE technique is a relatively new serologic test that
has been used for diagnosis of a variety of infectious diseases (Appendix A).
The CIE procedure is usually done in a laboratory using rather elaborate
electrophoresis devices.  For inclusion in this kit, the basic equipment re-
quired for electrophoresis has been miniaturized.  Thus, the CIE diagnostic
method may be applied to public health and tropical medicine problems in
remote locations that lack laboratory facilities.

Principle:  CIE tests may be used for detecting and identifying antigens,
such as bacteria, soluble bacterial products, or viruses.  CIE can also be
used to detect antibodies in the serum of convalescent or recovered patients.
The CIE test is an extension of agar gel diffusion, wherein antigen and anti-
body diffuse toward each other through an agar gel matrix.  When diffusing
antigen and homologous antibody meet, they form a visible precipitin line.
In CIE, direct electrical current is applied parallel to the line of dif-
fusion between wells cut in buffered agar on a slide.  This electrical cur-
rent forces the reactants in the wells toward each other and hastens the
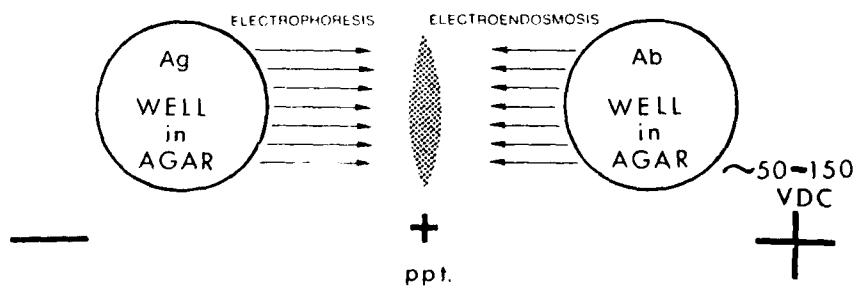diffusion process.  This results in a rapid, more sensitive precipitin test.
(Figure 5).



Figure 5:  In counterimmunoelectrophoresis, antigens move
toward the anode by electrophoresis and anti-
bodies move toward the cathode by electroendosmosis.

Figure 1: All equipment and supplies required for diagnostic tests (coagglutination, counterimmunoelectrophoresis, and microscopy) are carried inside a compact plastic case.
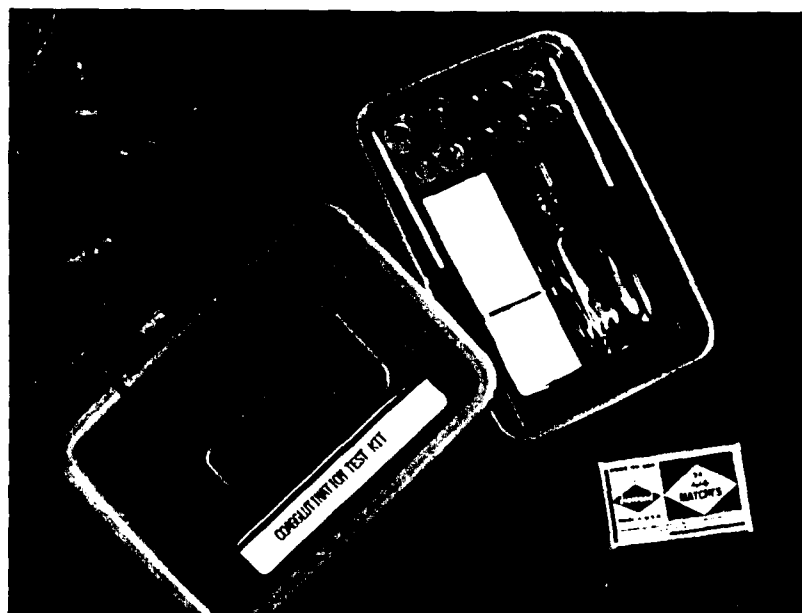


Figure 2: Materials for coagglutination or other types of slide agglutination tests are packed in a small sub-kit that may be used independently.
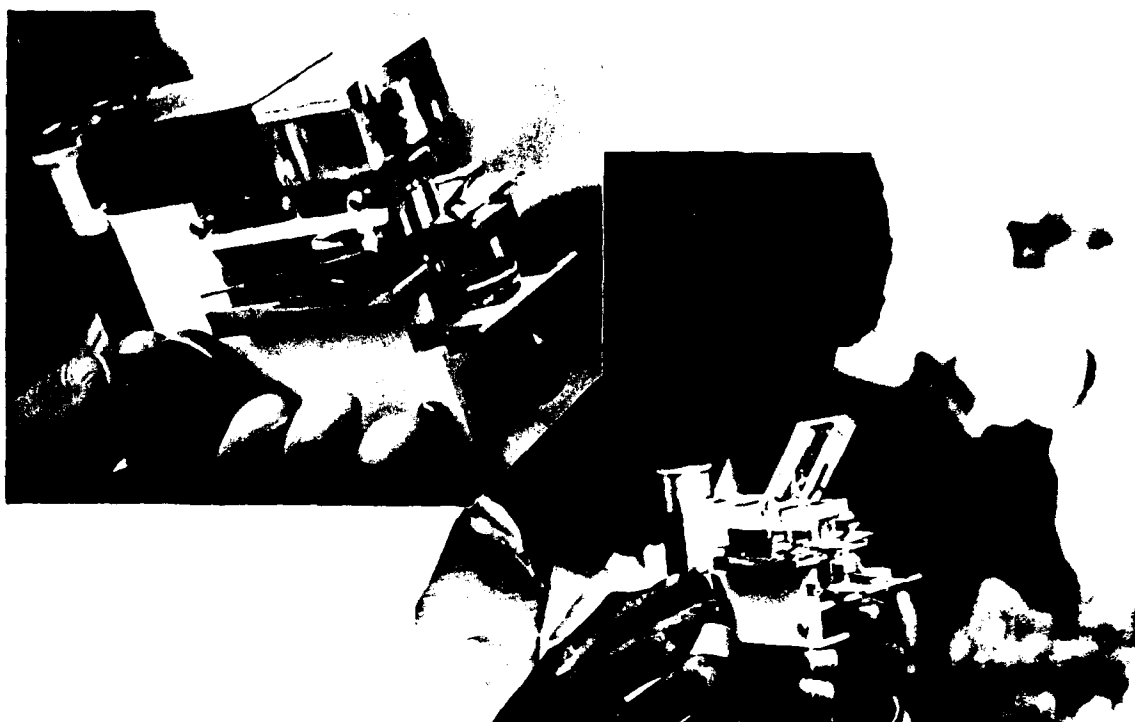
Figure 3: The McArthur Microscope is a rugged, compact field instrument offering full microscopy capabilities.
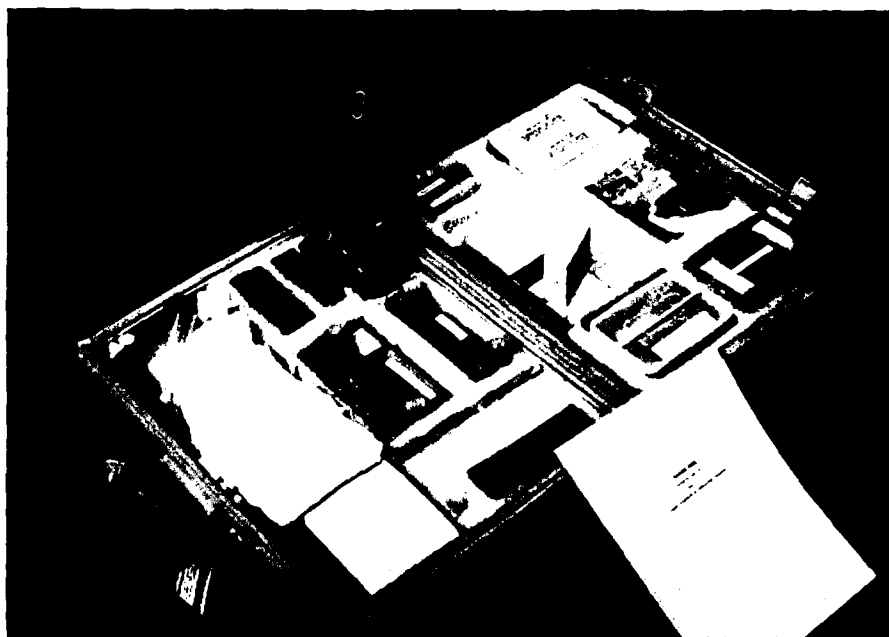


Figure 4: Dispenser bottles and insulated compartments hold stains and reagents for diagnostic tests.

The precipitin reaction in the CIE test is readily visualized using an oblique light against a dark background. It appears as a white line or arc within the agar gel matrix (Figure 6).



Figure 6: In a positive CIE test, a precipitin line forms in the gel on the slide.

Description: The CIE portion of the portable diagnostic kit is designed to perform all the operational and reagent preparation functions required for standard CIE tests. Design considerations include: reusable supplies, simplified operation, safety, protected portability, operation on 110 or 220 volt alternating current, operation from a 12 volt battery, and simplified test interpretation. Provisions have been included to chemically purify local fresh water and to prepare buffer solutions in the field. All necessary accessories such as pipettes, syringes, tubes, bottles, and slide preparation tools are contained in the kit. Thus, when the initial supply of reaction slides is exhausted, new supplies can be prepared in the field, permitting extended investigations.

Applications: The kit has been field-tested successfully for rapid, specific diagnosis of cerebrospinal meningitis. Performance tests have been made in West Africa using transport by both Land Rover and light aircraft to reach cerebrospinal meningitis outbreaks in remote rural areas.

To perform a CIE test in the field, the equipment is first removed from the carrying case (Figure 7). The electrical power source is then tested for suitability using the voltmeter provided (Figure 8). Cerebrospinal fluid (CSF) specimens are obtained from patients (Figure 9), the CIE test is set up (Figure 10), and diagnostic tests are performed (Figure 11). In this instance the work was done using a Land Rover fender as a laboratory bench. After 30 minutes the test may be read, and positive results are usually clearly seen in the agar gel as white precipitin lines (Figure 12).

The CIE test can be a significant aid to public health authorities for cerebrospinal meningitis control. The CIE test can transform a general clinical diagnosis of "meningitis" into an etiologically specific diagnosis; for example, cerebrospinal meningitis due to Neisseria meningitidis Gr. A. Reagents are available to specifically identify various N. meningitidis groups. Streptococcus pneumoniae, Haemophilus influenzae, and other etiologic agents. These distinctions are important to public health workers, since the recently available meningitis vaccines are immunologically specific. Thus, appropriate and timely vaccination may be given to "at risk" populations.

Field evaluations of the portable diagnostic kit were conducted in 1978, 1979, and 1980 in Upper Volta. The kit proved to be easily transportable: aboard commercial aircraft, in a light plane, in a Land Rover over rough tracks, and by hand. There was no damage to any component or reagent in the kit during 5 separate field operations. Additionally, kits were mailed from California to Cairo and air-shipped from California to Geneva. The cases showed evidence of rough handling, but internal components sustained no damage.

Figure 7: The equipment and supplies are removed from the case to perform meningitis diagnostic tests.
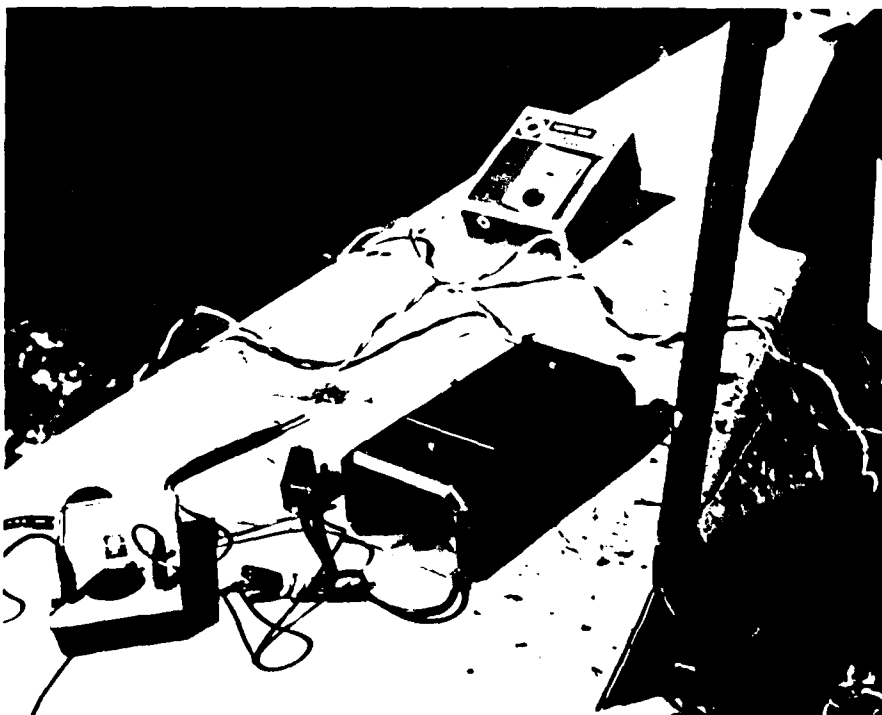


Figure 8: A multi-meter is used to test the electrical power source for suitability.

Figure 9: Cerebrospinal fluid specimens are obtained from meningitis patients by lumbar puncture.



Figure 10: The CIE apparatus is set up using a Land Rover fender for a laboratory bench.

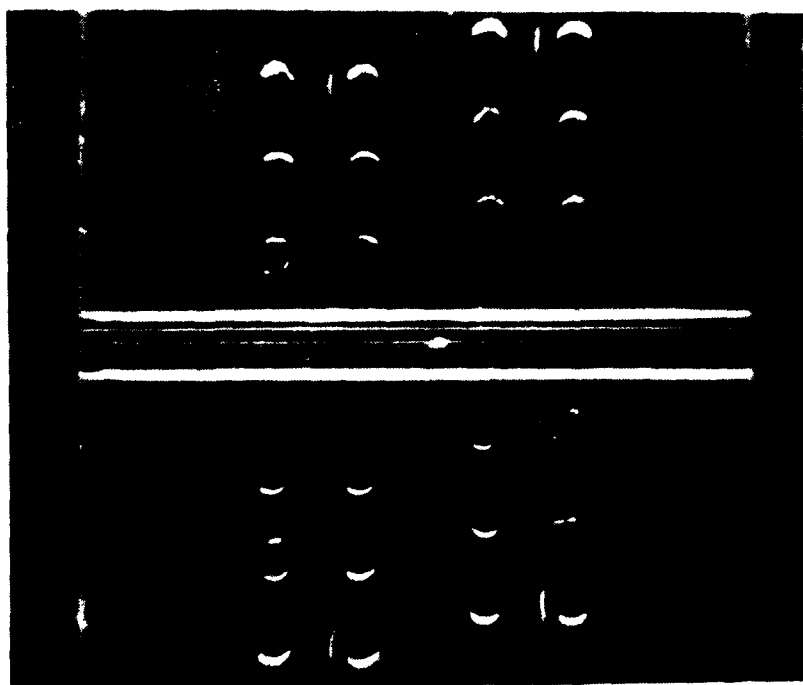Figure 11:  The CIE test requires about 30 minutes.



Figure 12:  Visible precipitin lines, slight arcs, indicate
positive tests for meningitis.

Examples of the value of the portable diagnostic kit in remote field situations are illustrated below. In the spring of 1979 the portable diagnostic kit was used to define the etiology of a cerebrospinal meningitis epidemic in Upper Volta. First, a trip was made by light aircraft to Fada N'Gourma in eastern Upper Volta. Among 14 CSF specimens obtained from suspected meningitis cases, 5 specimens were shown by CIE to contain Gr. C meningococcal antigen. This was the first evidence that this epidemic could be due specifically to Gr. C meningococci, rather than the more commonly found Gr. A.

Later, a second mission was mounted to confirm the epidemic etiology and to test an epidemic control system, i.e., rapid, specific diagnosis in the field followed by appropriate vaccinations. The rear seat and luggage compartment easily held the portable diagnostic kit, a portable jet injector, and 5000 doses of vaccine (Figure 13).



Figure 13: The aircraft is loaded with the portable diagnostic kit, vaccines, and a jet injector.

Flying to the general epidemic area in eastern Upper Volta, the plane was landed at dirt airstrips located near villages. Local medical authorities were contacted and queried regards cerebrospinal meningitis cases in their area. An on-going outbreak was discovered in Mahadaga. Cerebrospinal fluid specimens were obtained by lumbar puncture. CIE tests were set up and performed employing battery power from a local truck, since alternating current was not available.

Gr. C meningococcal antigen was found in 5 specimens, H. influenzae type b in 1, and S. pneumoniae in 1. Therefore, appropriate vaccinations were administered to local residents (Figure 14), and specific antimicrobial therapies were recommended for the various other types of infections (Figure 15).

While diagnostic CIE tests are routinely used in many European and North American hospitals, these studies are the first demonstrations of the application of CIE technology under field conditions.

Public health surveys also can be done using the CIE test. For example, it has been used for meningococcus throat carrier surveys, screening subjects for Gr. A and Gr. C meningococci (Figure 16). Results for CIE and conventional culture on split samples from 93 subjects were comparable, respectively 33% and 34% carriers.



Figure 16

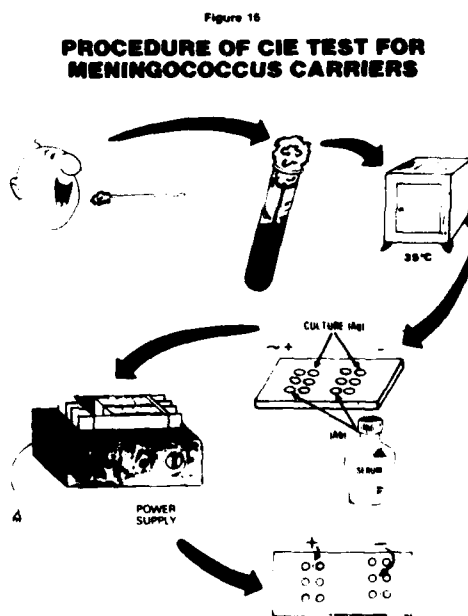**PROCEDURE OF CIE TEST FOR MENINGOCOCCUS CARRIERS**

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Figure 14: Meningitis protection for people at risk may be done using specific meningitis vaccines.



Figure 15: Rapid specific diagnosis permits prescribing appropriate therapy.

In this application, the CIE test was simpler, more rapid, and more cost-effective than doing a meningococcus throat survey by conventional culture (Table 1).

TABLE 1

COMPARISON of MENINGOCOCCUS CARRIER SURVEY METHODS

| FACTOR | CONVENTIONAL CULTURE | COUNTERIMMUNOELECTROPHORESIS |
|---|---|---|
| CULTURE MEDIA | 2-AGAR PLATES | 1-BROTH @ 2 ml |
| ELAPSED TIME | 56-60 HRS | 48-50 HRS |
| MAN HOURS | 13 | 3 |
| COST/100 TESTS: | | |
| LABOR @ $7.50/hr | $97.50 | $22.50 |
| MATERIALS | $92.18 | $34.67 |
| TOTALS | $189.68 | $57.17 |

CIE has been used to diagnose a variety of other infectious diseases, and applications for CIE have been developed in other areas as well (Table 2).

TABLE 2

RAPID DIAGNOSTIC APPLICATIONS of COUNTERIMMUNOELECTROPHORESIS (CIE)

| BACTERIOLOGY | MYCOLOGY | PARASITOLOGY | VIROLOGY |
|---|---|---|---|
| HAEMOPHILUS | ASPERGILLUS | AMEBIASIS | ARBOVIRUS INFECTIONS |
| MENINGITIS (Various) | BLASTOMYCES | MALARIA | GASTROENTERITIS |
| PNEUMONIA (Various) | CANDIDA | SCHISTOSOMIASIS | POLIOVIRUS |
| STAPHYLOCOCCUS | CRYPTOCOCCUS | TRICHINOSIS | SERUM HEPATITIS |
| STREPTOCOCCUS (Various) | HISTOPLASMA | TRYPANOSOMIASIS | SMALLPOX |

* * * * * * * * * * * * * * * * * * * * * * * *

OTHER COUNTERIMMUNOELECTROPHORESIS (CIE) APPLICATIONS

| ANTIBODY TITRATION | MICROBIAL TYPING | SNAKE BITE IDENTIFICATION |
|---|---|---|
| CANCER (HEPATOMA) | MYOGLOBINEMIA | TOXIN DETECTION |
| FORENSIC MEDICINE | PLANT VIRUS DETECTION | VACCINE CONTAMINATION CONTROL |
| HEART DISEASE | PUBLIC HEALTH SURVEYS | VETERINARY MEDICINE |

Thus, by incorporating appropriate antigens and antisera into the storage compartments of this portable diagnostic kit, it is possible to do public health surveys and epidemic diagnostic studies for many infectious diseases, when and where they occur, using the CIE test.

## COAGGLUTINATION (COAG) TEST

Background: In order to provide a broad range of rapid diagnostic tests, it is necessary to employ a variety of technical methods. In addition to microscopy and CIE, the COAG test has been included in the portable diagnostic kit because it can be used for certain diagnostic applications not possible using these others. Thus, by including both COAG and CIE tests in the portable diagnostic kit, its range of potential sero-diagnostic applications is expanded.

The COAG test offers a number of advantages. It requires only a minimum of equipment, and it is easy to perform. It is sensitive and specific. The reagents are relatively easily prepared, and they are inexpensive. Thus, the COAG test can provide an investigator with a practical tool for rapid diagnosis of certain infectious diseases.

Principle: The principle of the COAG test is quite different from that of CIE. In the COAG test, a killed and stabilized suspension of protein A-containing Staphylococcus aureus cells is employed as a carrier of specific antibodies, thus forming a "COAG reagent". To prepare a COAG reagent, an antiserum is mixed with killed, stabilized Staphylococcus cells. The antibody (IgG) becomes attached to the protein A coat via the Fc fragment of the IgG molecule, leaving the immunologically reactive Fab portion of the IgG molecule free to react with homologous antigen (Figure 17).

This "sensitized" staphylococcal COAG reagent is used in a simple agglutination-type test to specifically detect small amounts of antigens; for example those of Streptococcus, N. meningitidis, Salmonella, or Vibrio cholerae; in cultures or clinical specimens. The antigens act like a "glue" that binds antibody-sensitized staphylococci together. Thus, a matrix is formed (Figure 18). This reaction appears on the slide as clumps of cells (Figure 19).
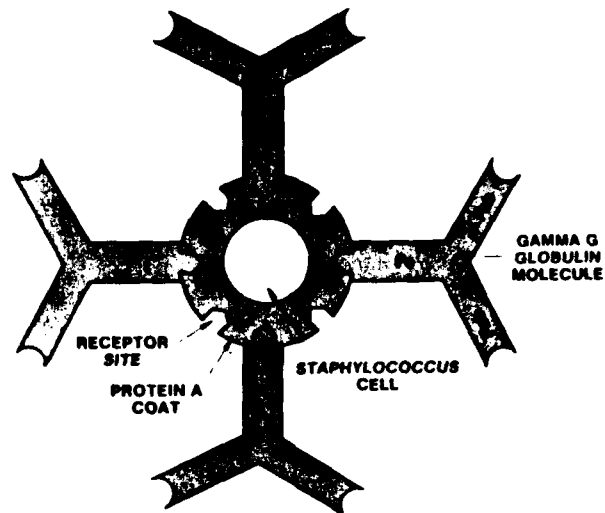
**COAGGLUTINATION REAGENT**

RECEPTOR SITE

PROTEIN A COAT

STAPHYLOCOCCUS CELL

GAMMA G GLOBULIN MOLECULE

Figure 17: When a stabilized staphylococcal cell is coated with antibody, it becomes a "coagglutination reagent".

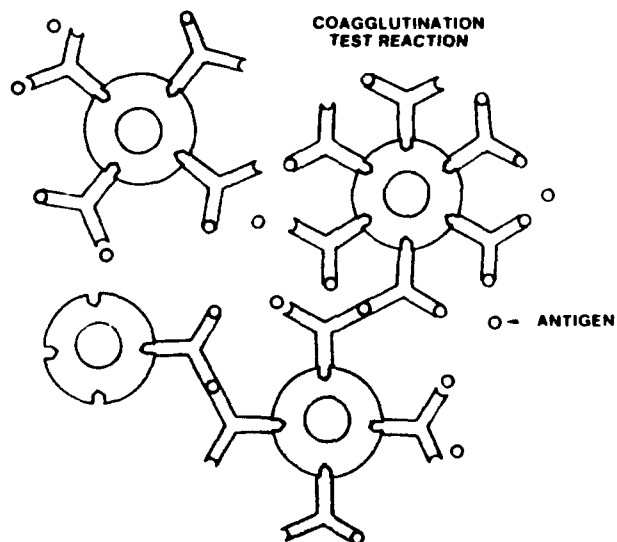COAGGLUTINATION TEST REACTION

O → ANTIGEN

Figure 18: Antigens react with specific antibodies on the staphylococcal cells, holding them together to form clumps.
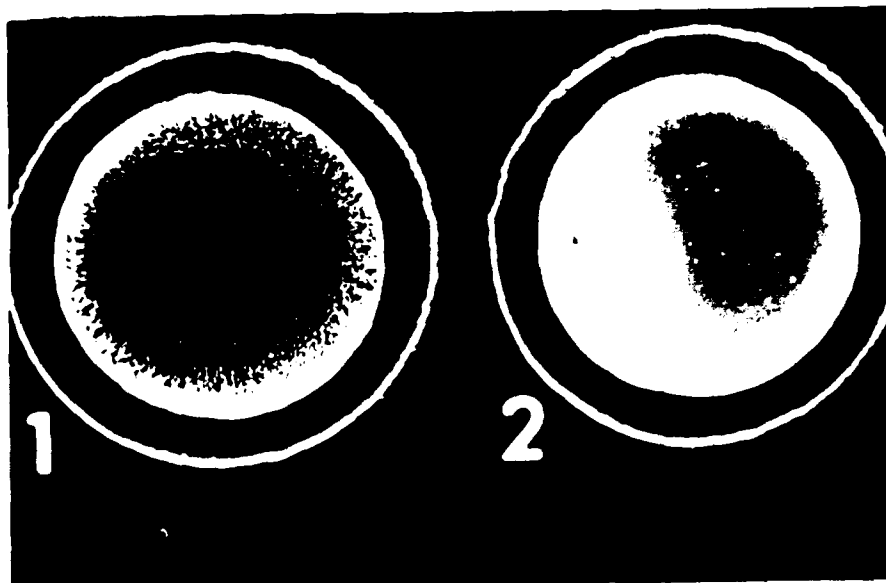
Figure 19: A positive coagglutination reaction appears on a slide as clumps or small granules of COAG reagent; positive test on the left.
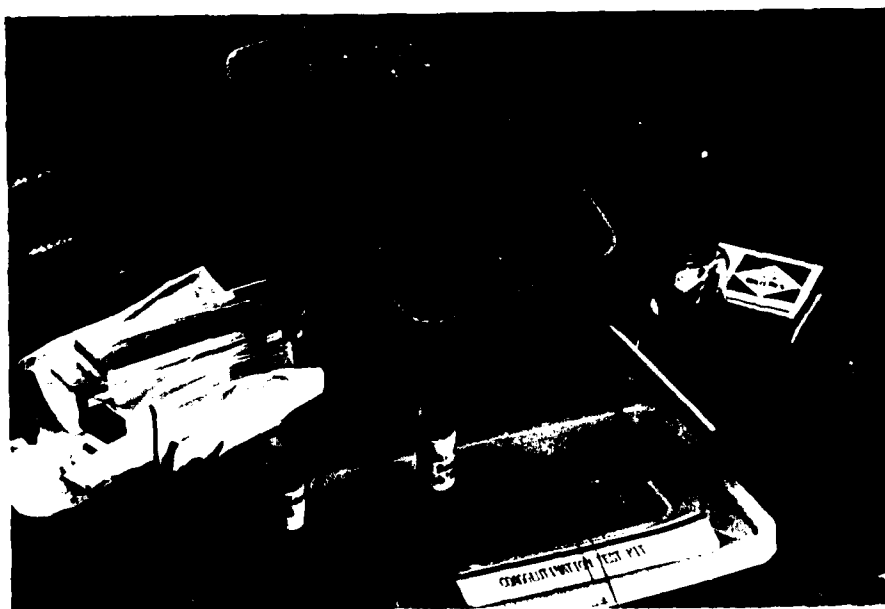


Figure 20: To do a COAG test, the materials are removed from the box and organized on a flat surface.
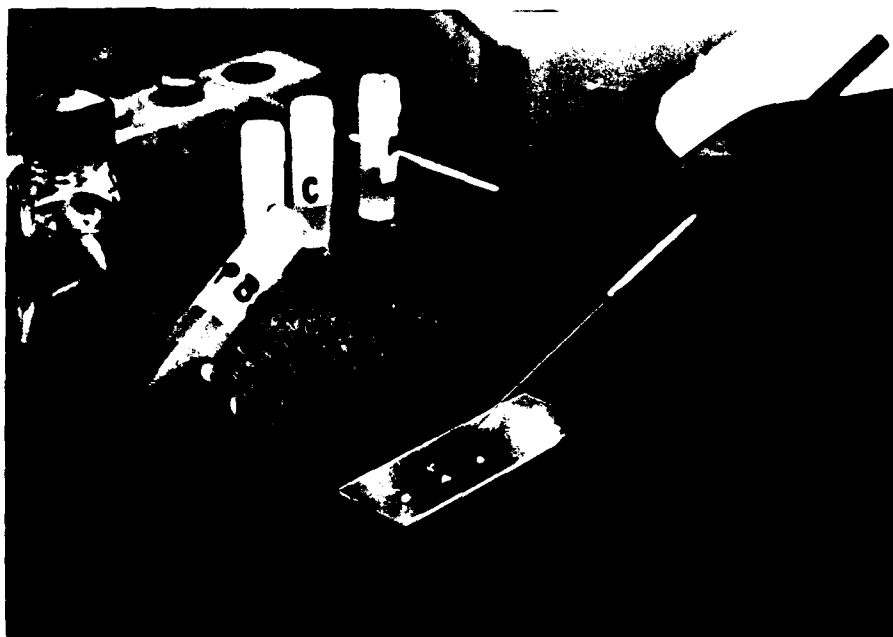
Figure 21: Droplets of the specimen and COAG reagent are placed on the microscope slide.



Figure 22: The droplets are thoroughly mixed by rocking the slide back and forth for about 1 or 2 minutes.

Description:  The COAG kit is contained in a foam-lined plastic box pro-
vided with compartments to hold the components.  These include small tubes
containing the COAG reagents and control antigens, bacteriological loops, an
alcohol burner, and microscope slides.  This small box is incorporated into
the primary portable diagnostic kit as a component, but it may be removed
and easily carried on the person to be used independently.

In field practice, any flat surface can serve as a work area (Figure 20).
The COAG test is easily performed in almost any situation.  First, droplets
of the liquid specimen (cerebrospinal fluid, "rice water" stool, broth
culture, etc.) are placed on a microscope slide using the bacteriological
transfer loop (Figure 21).  Then a drop of COAG reagent is mixed with the
specimen, and the slide is rocked back and forth to make the reaction occur
(Figure 22).  After about 1 minute, positive results will be observed as a
clumping, clearing, or granulation of the smooth, milky reagent (Figure 19).

Applications:  Although the COAG test is a relatively new method, a
variety of practical applications already have been published.  These are
both for rapid diagnosis and also for identification and typing of bacterial
cultures (Table 3).

TABLE 3

APPLICATIONS of the COAGGLUTINATION (COAG) TEST

| A. RAPID DIAGNOSIS | B. BACTERIAL IDENTIFICATION/TYPING |
|---|---|
| CEREBROSPINAL MENINGITIS | ESCHERICHIA COLI ENTEROTOXIN |
| CHOLERA | MYCOBACTERIUM |
| PNEUMOCOCCAL PNEUMONIA | NEISSERIA GONORRHOEAE |
| SALMONELLA ENTERITIS | SALMONELLA |
| STREPTOCOCCAL INFECTIONS | SHIGELLA |
| TYPHOID FEVER | STREPTOCOCCUS |

An example of a public health application for rapid diagnosis using the COAG test is provided by studies done in Jakarta, Indonesia. A rapid diagnostic technique was developed to aid in control of a salmonellosis epidemic in a hospital nursery. The technique involved overnight culture of stool specimens in a selective enrichment broth, followed by specific detection of Salmonella antigen in those cultures using the COAG test.

Salmonellosis diagnosis was both sensitive and specific by this enrichment-COAG test. In comparison with conventional culture procedures, employing both direct and enriched isolation methods, enrichment-COAG identified 95% of the culturally positive cases. The COAG test detected one symptomatic case that was not detected by conventional culture (Table 4). Specificity control studies were made with 50 stool specimens from normal infants in a nursery that was free of enteric infections. These were all negative by both methods.

TABLE 4

COMPARISON of CONVENTIONAL CULTURE and ENRICHMENT BROTH-COAGGLUTINATION TESTS

for DIAGNOSIS of SALMONELLOSIS

| Final Diagnosis | Specimens | Positive Results | |
| --- | --- | --- | --- |
| | | Culture | Coagglutination |
| Salmonella oranienburg | 87 | 86 | 83 |
| Negative | 75 | 0 | 0 |

The enrichment culture-COAG method provided a more rapid diagnosis than conventional culture. Salmonellosis was diagnosed in about 3 to 4 days by conventional methods, while the enrichment culture-COAG method yielded diagnoses in about 18 hours.

The enrichment culture-COAG method was more cost-effective than conventional culture. While diagnostic results were about equal, the COAG method only cost 1/5 as much (Table 5). This savings would permit expanded programs for public health laboratories and significantly reduce the cost of carrier surveys.

TABLE 5

COMPARISON of METHODS for PROCESSING 100 FECAL SPECIMENS

for SALMONELLAE or VIBRIOS

| FACTOR | CONVENTIONAL CULTURE | ENRICHMENT-COAGGLUTINATION |
|---|---|---|
| ELAPSED TIME for TESTS | 3.5 DAYS | 1 DAY |
| TECHNICIAN BENCH TIME | 16 HOURS | 3 HOURS |
| LAB AIDE SUPPORT TIME | 6 HOURS | 1 HOUR |
| PERSONNEL COSTS: | | |
| Technician @ $7.50/hr | $120 | $23 |
| Lab Aide @ $4.00/hr | 24 | 4 |
| CULTURE MEDIA COSTS | 29 | 2 |
| TOTAL COSTS | $173 | $29 |
| ANTISERUM USE (1 ml) | 20-50 TESTS | 1000 TESTS |

Another enrichment culture-COAG test was developed to detect and identify V. cholerae. This was tested in parallel with conventional methods during survey studies in Bahrain for V. cholerae carriers among cholera case contacts and food-handlers. The cholera enrichment-COAG test detected 75% of those carriers detected by conventional methods. The cost of this enrichment culture-COAG method was much less than conventional culture methods.

Sensitivity of a COAG reagent is directly dependent on the potency of the antiserum used. Using very potent antisera, COAG reagents have been prepared that will detect V. cholerae by direct tests of fecal specimens from acute, severe cholera cases. Thus, using the COAG test, specific rapid diagnosis of acute cholera can be made within a few minutes.

Stability of COAG reagents appears to be quite good. COAG reagents are stable for at least 8 weeks at either 25 or $37^{\circ}$C and for at least 6 months in the refrigerator. Concentrated COAG reagents have been mailed internationally, sealed in plastic envelopes. These have performed satis-factorily after being 4 weeks en route.

## CULTURE INCUBATION CAPABILITY

In tropical areas bacteriologic cultures may often be made without using an incubator, but in other places an incubator is needed. A miniature waterbath-incubator has been included in the kit. This is useful in the selective enrichment culture-COAG tests for diagnosis of salmonellosis or cholera. Selective enrichment-COAG tests also may be used in public health surveys for detecting carriers of enteric pathogens. The miniature incubator-waterbath can be operated from various electrical power sources.

## SUMMARY

A compact portable diagnostic kit has been described that has been de-signed for diagnosis of infectious diseases and detection of asymptomatic carriers of pathogenic bacteria. The kit capabilities include diagnostic microscopy, counterimmunoelectrophoresis (CIE), coagglutination (COAG), and culture incubation. The electrical apparatus included can be operated from a variety of power sources. The COAG test materials are contained in a small sub-kit that may be used independently. All these capabilities may be em-ployed for rapid diagnosis of a wide variety of infections caused by dif-ferent pathogenic organisms. Field tests, wherein meningitis, salmonellosis, and cholera have been diagnosed in epidemic situations, have demonstrated the practicality and appropriateness of this portable diagnostic kit for use where laboratory facilities do not exist. Thus, this field kit has broad potential for rapid, low cost diagnostic and survey applications in public health and tropical medicine.

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**ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE**

# Ordinary Meeting

**Manson House, Thursday, 11th December, 1975**

*The President:* DR. C. E. GORDON SMITH, C.B., F.R.C.P., F.R.C. PATH., *in the Chair*

**Symposium on recent advances in the serology of tropical disease**

Order of speakers: DR. C. C. DRAPER, DR. A. VOLLER, PROFESSOR P. AMBROISE-THOMAS

**Discussion:** PROFESSOR BRUCE-CHWATT
PROFESSOR AMBROISE-THOMAS
DR. I. A. McGREGOR

# The use of counterimmunoelectrophoresis in immunodiagnosis

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In many infectious diseases in which it is difficult always to find the responsible organisms immuno-diagnostic tests can be of great value. Used for many years by virologists and bacteriologists they are being increasingly applied to tropical parasitic infections. In addition to their use in clinical medicine such tests are being more and more recognized as helpful in epidemio-logical studies, in that they may often give information on the period prevalence of an infection (e.g. DRAPER and VOLLER, 1972; VAN DER KAAY, 1975). The variety of such tests shows that none is generally applicable. Counterimmunoelectrophoresis (CIE) is a recent develop-ment, still in an experimental stage.

### Principles of the CIE test

Many different types of antibody are produced in response to an antigenic stimulus, as in an infection, including precipitating antibodies. The latter can often be shown in a gel diffusion system in which antibodies and antigens are allowed to diffuse towards each other from wells cut in a slide or plate coated with agar. The method is notoriously insensitive and slow; some of the mechanical reasons for this are illustrated in the top part of the Figure. The reactants diffuse in all directions and only the small amounts meeting in the area between the wells can form precipitation lines. The middle part of the Figure shows the effect of passing an electric current through the plate or slide. Most antigens have an electro-phoretic mobility which causes them to move towards the anode. If a buffer of about pH 8.0 is used in the agar the immunoglobulins are electrophoretically neutral. How-ever electroendosmotic currents occur in the agar which carry the immunoglobulins towards the cathode. Both antigens and antibodies move with a narrow front so that maximal amounts of each are driven towards each other, to react and form a line. This increases both the speed and sensitivity. The bottom part of the Figure shows a double system: the specimen for test, which may contain antibody or antigen or both, is placed in a central well with the antigen on the cathodal side and a specific antiserum on the anodal side.

The basic materials and apparatus needed for a CIE test system are simple and cheap. Many variations are possible. For example, by using the purer agarose or mixtures of agar and agarose the mobility of the reactants can be changed empirically to determine the most suitable (KELKAR and NIPHADKAR, 1974). The same effect can be obtained with a discontinuous buffer system, using buffers of different molarities in the agar and in the electrode compartments. Different sizes, shapes and spacings of the wells can be used, the temperature and electric current can be varied, and agents such as dextran, or stains, can be used to enhance the precipitin lines. Many of these variations are described by CROWLE (1973), and the references given in the following sections have been chosen to show the variations in technique that are possible. From these it is apparent that no one system is generally applicable and the most suitable details must be determined by experiment.

### Applications of the CIE test in tropical and other infections

*Virus infections*

One of the earliest uses of the CIE test was for the detection in blood of the surface antigen of the agent associated with hepatitis B and of antibody to it (e.g. WHO, 1970; PRINCE and BURKE, 1970; GOCKE and HOWE, 1970; WALLIS and MELNICK, 1971; COMBRIDGE and SHAW, 1971). It has, however, been superseded by the more sensitive tests of radioimmunoassay, indirect haemagglutination (IHA) and ELISA. Descriptions have recently been given of the use of the method in other virus infections, for example for the detection of both the viral antigens and antibody in dengue haemorrhagic fever (CHURDBOONCHART et al, 1974), and of antibody in California virus infection (BALFOUR and EDELMAN, 1974) and in influenza (BERLIN and PIROJBOOT, 1972), and it has been used as an aid for the typing of adenoviruses (HIERHOLZER and BARME, 1974). For the detection in stools of a reovirus-like agent associated with infantile gastro-enteritis the method was rapid but less sensitive than electron microscopy or a complement fixation test
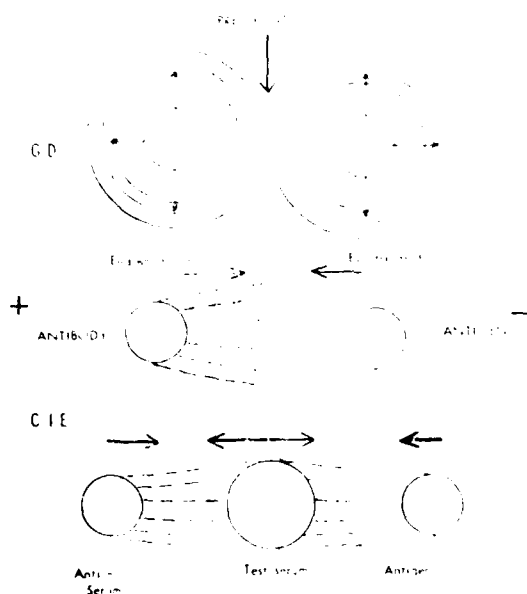
Fig. Principles of gel diffusion (GD) and of counter-immunoelectrophoresis (CIE).

(SPENCE et al, 1975). It is possible that the method may have scope in the future for the rapid diagnosis of other viral infections.

### Bacterial infections

For some of these the method is rapid and sensitive and has been found to be of value. In meningitis it is of clinical and of epidemiological interest to determine the responsible organism. Stained smears of cerebrospinal fluid (CSF) are often negative while cultures take time. CIE is a rapid and sensitive method for the detection in CSF and serum of the soluble polysaccharide antigens of *Neisseria meningitidis, Diplococcus pneumoniae,* and *Haemophilus influenzae* (EDWARDS, 1971; EDWARDS et al, 1972; COONROD and RYTEL, 1972). The method allows the detection within hours of nanogram amounts of antigen and is an aid to rapid specific diagnosis in clinically suspect cases, especially those in which the start of chemotherapy makes culture difficult. The amount of antigen detectable by CIE, estimated by reacting dilutions of CSF against specific antisera, correlates with the clinical course and prognosis as, also does the detection of circulating antigen in serum. In the meningitis belt of West Africa the CIE method has been extensively and successfully used for clinical and epidemiological purposes and it has been shown that meningococcal antigens can be detected in samples of CSF collected in the field on absorbent paper (GREENWOOD et al, 1971; GREENWOOD and WHITTLE, 1974a and b; WHITTLE et al, 1975a; ERWA et al, 1973). In one study CIE was also used to detect antibody in convalescent patients (WAHDAN et al, 1973). It has recently been used to show a shift of the predominant antigenic group of meningococcus from A to C (WHITTLE et al, 1975b). A further development of the test has been its use for detecting similar bacterial antigens in cases of pneumonia in the serum, sputum,

effusions, or urine (COONROD and RYTEL, 1973; BARTRAM et al, 1974; LEGGETT and GREENWOOD, 1974, 1975; VERHOEF and JONES, 1974). Reports have been made of the use of the method for detecting antibodies to *Treponema pallidum* (FULLER and TAKAHASHI, 1972) but the sensitivity is probably less than that of traditional methods.

### Mycoses

CIE has been extensively used to detect antibody in mycotic infections, one of the first of these uses being in systemic candidiasis (REMINGTON et al, 1972). It has since been used to detect antibodies in a variety of such infections and has become established as a useful and rapid diagnostic tool (MACKENZIE and PHILPOT, 1975). Attempts to detect circulating fungal antigens have been inconclusive.

### Protozoal infections

In malaria, simple gel diffusion techniques have been used with notable success to demonstrate the presence of a variety of circulating antigens and of antibodies to them in the sera of heavily infected people and animals (McGREGOR and WILSON, 1971; WHO, 1974, 1975). However, preliminary attempts to increase sensitivity by using CIE to detect circulating antigens and antibodies have been more encouraging in rodent malaria infections (ZAMAN et al, 1972; SEITZ, 1975) than in infections in monkeys with human parasites (BIDWELL et al, 1973; BIDWELL and VOLLER, 1975). As a method of detecting total antibody for epidemiological studies in malaria the CIE technique is probably inferior to immunofluorescence (AMBROISE-THOMAS, 1976), indirect haemagglutination, or the ELISA method (VOLLER, 1976) but may be worthy of further development for more detailed immunological studies.

It has been possible to detect antibodies in infections with *Trypanosoma cruzi* and *Leishmania donovani* with CIE (AFCHAIN et al, 1970; DESOWITZ et al, 1975), but, using only crude antigens, the method was less sensitive than immunofluorescence. In a separate trial in South America CIE was considerably less sensitive than complement fixation and immunofluorescence tests in detecting antibodies to *T. cruzi* in a human population (MOTT, personal communication).

More encouraging results for the use of the CIE tests in a protozoal infection have been reported by KRUPP (1974) who employed it to detect antibodies in amoebiasis. Using an antigen prepared from sonicated *Entamoeba histolytica* she found that the test was more sensitive than simple gel diffusion and compared favourably in sensitivity and specificity with the more complicated IHA test. The number of precipitin arcs obtained in CIE was proportional to the titre in the IHA test, and both of these were greater in those with hepatitis than in those with dysentery alone. However, none of the tests could differentiate antibodies present in an active infection from those persisting after treatment.

### Helminth infections

A problem in all immunodiagnostic tests for helminth infections is the complexity of the antigens involved. In general little is known of those fractions of the various stages of helminths, or their secretions, which are immunogenic in the host. Therefore the great majority of such tests suffer from lack of sensitivity and/or of specificity. However, there have been some encouraging reports of the use of CIE.

Using a fine particle-associated antigen from mature

larvae of *Trichinella spiralis*, DESPOMMIER et al (1974) found the CIE test to be more rapid, sensitive and specific than the gel diffusion test and probably more specific than flocculation tests. Because of the long persistence of precipitating antibodies in this infection the test may be useful for epidemiological surveys.

In hydatid disease it has been claimed that CIE gives greater sensitivity as well as speed when compared with the standard gel diffusion test for antibodies, hydatid fluid being used as antigen (KELKAR and KOTWAL, 1975). Using crude antigens prepared from adult and micro-filarial stages of *Dirofilaria immitis* the CIE method has recently been used to demonstrate precipitating antibodies, specific for these stages, in animals infected with this parasite and in humans with Bancroftian filariasis (DESOWITZ and UNA, personal communication). The disappearance of microfilariae from the blood seems to be associated with the development of precipitating antibody to that stage, and it is thought that the test may be developed as a useful adjunct for diagnosis.

In attempts to produce a much needed improved immunodiagnostic test for schistosomiasis, PHILLIPS and DRAPER (1975a) also used the CIE technique. There is growing evidence from observations on the immuno-pathology of the disease, and from study by various methods of sera from infected animals and humans (BRITISH MEDICAL JOURNAL, 1975), that antigens liberated by the worm or ova may be present in the circulation in the form of antigen-antibody complexes. Their presence may contribute to some of the anomalous results in serological tests for schistosomiasis. Therefore a double CIE system was used, with the test serum in the central well, an anti-schistosome serum in the anodal well (see Figure) to detect any antigen in the serum which could be expected to migrate anodally, and a prepared schisto-some antigen in the cathodal well to detect antibody in the immunoglobulins moving cathodally. The antigen was prepared by affinity chromatography using antiserum from a naturally infected animal as an immunosorbent (PHILLIPS and DRAPER, 1975b). This method, more direct than chemical fractionation, has given antigens active in the CIE which have also been used in the ELISA test (VOLLER, 1975), but the method is not yet entirely reproducible and the yield of reactive antigens may be low. Using acidification of the sera to dissociate possible antigen-antibody complexes before testing by CIE it was at first thought that it was possible to detect circulating antigen in a considerable proportion of individuals from tropical areas where *Schistosoma mansoni* infection is hyperendemic, but subsequent use of this method has shown that in fact many of the precipitin arcs on the anodal side were due to non-specific effects of the acid. However, in a small proportion of people, about 2% of those tested, all with heavy egg loads, additional arcs were seen which may have been due to circulating antigen. The detection of antibody lines on the cathodal side was more specific and Table II shows the results of tests, with one batch of reactive antigen, of capillary blood samples from an area where *S. mansoni* is hyperendemic. The higher prevalence of antibodies than ova in the youngest age group suggests that infection may be higher in them than shown by parasitological examination. If this test can be made reproducible it could be useful as a field test for measuring schistosomiasis incidence and prevalence particularly at the lower levels, as, for example, in a control scheme when it is important to get accurate information.

Table I   Some uses of CIE (CEP, IEOP) in diagnosis of tropical diseases

|  |  | Detection of: | |
|---|---|---|---|
|  |  | Ab | Ag |
| Viruses | HBsAg, dengue, reovirus | · | |
|  | Flu*, California | : | |
| Bacteria | N. meningitidis, H. influenzae, | | |
|  | D. pneumoniae, Pseudomonas | | |
| Mycoses | Candida | : | |
| Protozoa | Malaria | · | · |
|  | E. histolytica, T. cruzi, | | |
|  | Leishmania | | |
| Helminths | Trichinella, hydatid, filaria | · | |
|  | Schistosoma | · | ? |
| Misc. | Snake bite | | · |

Table II - Gezira, Sudan. Antibodies to *S. mansoni* by CIE and excretion of *S. mansoni* ova

| Age in years | Number examined | % with antibody | % excreting ova |
|---|---|---|---|
| 1-4 | 101 | 48 | 11 |
| 5-19 | 643 | 96 | 80 |
| >20 | 275 | 99 | 74 |

*Other applications*

CIE has been used for the detection of alpha foeto-protein (KOHN, 1970) but has been superseded by more sensitive radioimmunoassay procedures. An interesting application in tropical medicine has been its use for rapid identification of snake venoms in wound aspirates, blister fluid, or urine (GREENWOOD et al, 1974). This is of some clinical value in cases of snake bite when the species is unknown.

**Conclusions**

The CIE test is rapid, does not need expensive apparatus, and is easy to perform if good specific antigens and antisera are available. It has been shown to be of undoubted value in the diagnosis of some bacterial infections by detection of antigens in body fluids and may be of value in virus and fungal infections. For protozoal and helminthic infections, in those instances where it has been tried, it is usually less sensitive than other techniques for the detection of antibodies, but the production of more reactive and specific antigens may improve this. One disadvantage is that the class of antibody cannot be ascertained as in IFA or ELISA tests. A possible advantage is that it may show certain qualitative as well as quantitative differences—the position and shape of different precipitin lines may correlate with the amount of infection and immune status of the host. With improvements in technique, it may be of value as a method for detecting circulating antigens in some infections.

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# Appendix B

## A Bibliography of Diagnostic Applications of the Coagglutination Test

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

CIVAPP: Rapid Diagnosis; Portable Kit: Methodology;
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

(U) A portable kit has been developed for identifying infectious disease agents under field conditions, i.e., without laboratory facilities. The basic techniques employed are counterimmunoelectrophoresis, coagglutination, and diagnostic microscopy, including differential stains. This portable kit has been evaluated in various field and laboratory circumstances in tropical and temperate areas for rapid diagnosis of cerebrospinal meningitis, salmonellosis, and cholera. The carrying case, working components, and diagnostic reagents

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have withstood aircraft transport, baggage handling, international mailing, international air freight shipment, and rough road transport by Land Rover.  It has performed at ambient temperatures ranging from $15^{\circ}$ to $43^{\circ}$C.  By simply substituting other appropriate antisera, antigens, and reagents, this system is adaptable to diagnostic identification of numerous other infectious disease agents.